J.C. Maillard [1]
C. Palin [2]
I. Trap [3]
A. Bensaid [3]

An attempt to identify genetic markers of resistance or susceptibility to dermatophilosis in the zebu Brahman population of Martinique

MAILLARD (J.C.), PALIN (C.), TRAP (I.), BENSAID (A.). Une tentative d'identification de marqueurs génétiques de résistance ou de sensibilité à la dermatophilose dans la population de zébus Brahman de la Martinique. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 291-295

La dermatophilose est une maladie associée à la tique *Amblyomma variegatum* ; une prédisposition génétique à la manifestation des symptômes a été démontrée. En effet, les bovins Créole de la Guadeloupe constituent une population très résistante à cette maladie, tandis que les zébus Brahman de la Martinique semblent très sensibles. Néanmoins, dans cette population de Brahman il y a un gradient en ce qui concerne l'intensité des symptômes, selon les individus. Dans plusieurs troupeaux de ces zébus Brahman purs, maintenus dans les mêmes conditions, des groupes d'animaux sensibles et résistants ont été sélectionnés sur la base de la présence simultanée d'animaux cliniquement affectés par la dermatophilose et d'animaux non affectés. Plusieurs systèmes génétiques très polymorphes ont été étudiés sur ces animaux, tels que l'hémoglobine, l'albumine, le complexe BoLA (classes I et II) et le gène de gamma S crystalline. Seulement l'exon 2 du gène BoLA-DRB3, examiné par la technique de PCR-RFLP a montré un polymorphisme intéressant. Une carte génotypique a été établie qui montre au moins 4 allèles différents, dont un semble particulier à un animal sensible à la dermatophilose. Avant d'arriver à des conclusions, d'autres recherches avec plus d'échantillons d'ADN d'animaux sensibles sont nécessaires.

Mots clés : Zébu Brahman - Dermatophilose - Tique - *Amblyomma variegatum* - Résistance aux maladies - Gène - Polymorphisme génétique - Marqueur génétique - Martinique.

INTRODUCTION

Skin disease associated with the actinomycete bacterium, *Dermatophilus congolensis,* has been recognized since 1910 in Africa where it was first described in the Congo by VAN SACEGHEM (28). Since then, the disease continues to seriously limit animal production in the tropics and remains a problem in the temperate regions. It has been a particularly severe cause of losses in the Caribbean (26) where it has forced farmers in some areas to abandon ruminant husbandry. Although the epidemiology of dermatophilosis is well known, the pathogenesis of the disease is still poorly understood. No method for control, which is readily applicable in the areas of extensive husbandry where it is most needed, has yet been found. Nevertheless, recent research has begun to provide explanations for some of the factors which increase the severity of diseases (13, 14) and particularly dermatophilosis.

Resistance to infection differs considerably both between species (11), within species and between breeds (2, 3, 5, 10, 20). Individual variation within breeds is also quite significant.

Amongst cattle, the N'Dama and Muturu breeds are highly resistant (20), whilst European *Bos taurus* breeds are particularly susceptible, as are *Bos indicus* like Brahman cattle (5, 10). In the Caribbean, the Creole crossbreed cattle appear to be highly resistant (2, 3). This character seems innate and probably acquired because of its African *Bos taurus* N'Dama origin (17), maintained since its establishment in the West Indies by the constant pressure of the "Senegalese" tick *Amblyomma variegatum.* Creole cattle crossbred with susceptible breeds such as European cattle or Brahman zebus show a decrease of the resistance. The zebu Brahman population of Martinique, is mainly susceptible. It is a stabilized mix of Indian zebu breeds (Gir, Nellore, Gujera...) made in the USA (18) and introduced in Martinique around 1952. Being since in contact with the tick *Amblyomma variegatum*, there exists in these susceptible cattle a gradient in the individual susceptibility to dermatophilosis (2).

MATERIAL AND METHODS

Several herds of pure zebu Brahman were selected because of the simultaneous presence of animals affected or not by clinical dermatophilosis. In these herds, 121 non-related animals were classified as resistant or susceptible according to the absence or presence of skin lesions. Sixty nine (69) of them, highly susceptible, showed severe skin lesions whereas the 52 others never showed lesions after one year of continous survey. It is important to notice that all animals belonging to the same herd were kept under the same farming conditions.

On these animals was studied the following panel of polymorphic marker systems:

- the B chain of haemoglobin which is an erythrocytic protein showing 10 electrophoretic variants by electrophore-

1.CIRAD-EMVT, BP 1232, 97185 Pointe-à-Pitre Cedex, Guadeloupe.

2. Direction des services vétérinaires, Martinique.

3. CIRAD-EMVT, 10 rue Pierre Curie, 94704 Maisons Alfort Cedex, France.

sis on cellulose acetate (1). Only the 2 main alleles A and B were detected ;

- the serum albumin, also analysed by PAGE (6), showing 7 electrophoretic variants. As for haemoglobin, only the 2 main alleles F and S were detected ;

- the bi-allelic polymorphism of the gamma S crystallin gene, investigated by molecular analysis (9). This gene shows a punctual G/C substitution detected at the base number 1754. After amplification by the PCR technique and separation by agarose gel electrophoresis, 2 DNA fragments of 128 and/or 149 bp can be detected. Each DNA fragment is representative of one of the two alleles ;

- the BoLA complex (25), highly polymorphic, shows in cattle about 50 allo specificities in the class I region (4). These are serologically detected by the standard method of lymphocytotoxicity. Some antigens of the BoLA class I region are associated with resistance or susceptibility to several diseases (21) such as eye cancer, mastitis (19, 24) or leucosis (12);

- the polymorphism of BoLA class II genes (8, 15), is investigated in the exon 2 of the BoLA-DRB3 gene (7, 22, 23), (at least 50 alleles) using the PCR-RFLP technique (16, 27). Genomic DNA of 1 susceptible and 7 resistant animals was obtained from nucleated blood cells by the standard method of phenol-chloroform extraction. After amplification by PCR, using primers surrounding the exon 2 of the BoLA-DRB3 gene, we divided the amplified product in 3 aliquots each one being separately digested by one of the following restriction enzymes *HaeIII, DpnII* and *Fnu4hI*. The DNA fragments were separated by agarose gel electrophoresis.

RESULTS AND CONCLUSIONS

No significant differences in allele frequencies between resistant and susceptible animals were found either in the polymorphic protein systems or in the gamma S crystallin gene (table I).

Only the BoLA system seems to be of interest. The W6 class I specificity showed a different frequency but the statistical significance is low (P <9 %).

On the other hand, an extensive polymorphism was detected (fig. 1) within exon 2 of the DRB3 gene.

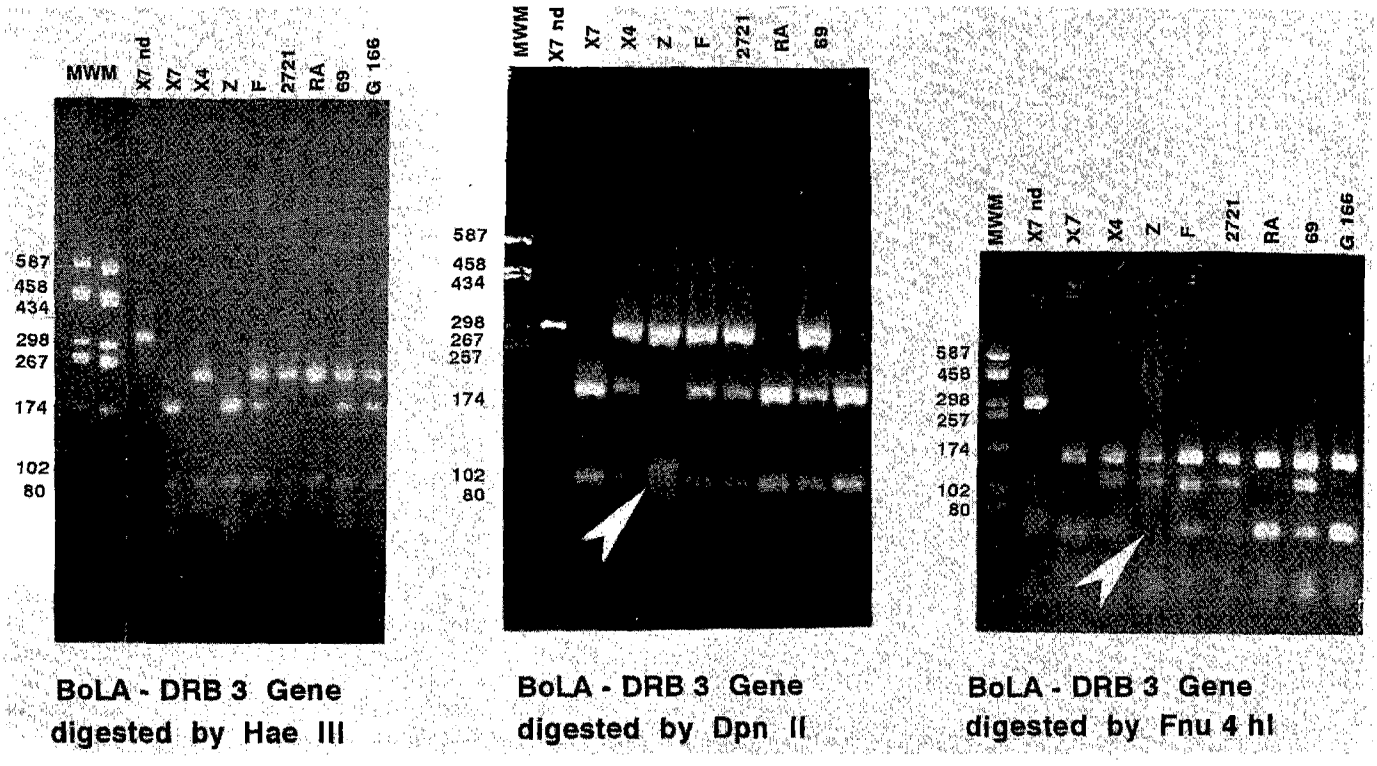

Figure 1 : Electrophoretic polymorphism of exon 2 of the BoLA DRB3 gene revealed by restriction fragment length polymorphism (RFLP). After PCR amplification, DNA of seven resistant animals (X7, X4, F, 2721, RA, G166 and 69) and one susceptible animal (Z) were subjected to restriction enzyme digestion (HaeIII, DpnII *or* Fnu4hI) *and run in a 2.5 % agarose gel. Size determination of the DNA fragment was performed by comparing samples to the products of the plasmid PUC 19 digested by* HaeIII *(MWM). Note in lane Z of the* DpnII *pattern the 3 low molecular weight DNA fragments (around 100bp) and in the* Fnu4hI *pattern, the absence of a 60bp DNA fragment characterizing this susceptible animal.*

TABLE I Allele frequencies (Fq) are expressed in percentage (%) of the Brahman population tested.

		Resistant		Susceptible		Diff.
		Nb	Fq	Nb	Fq	
HBB	A B	52	55.5 45.5	69	55 45	NS
ALB	F S	52	11.8 88.2	69	12.9 87.1	NS
G.S. CRYST.	128 bp 149 bp	9	44.4 55.6	9	55.6 44.4	NS
BoLA W6		52	4	69	10	P=0.09

Nb : number of animals tested, HBB : haemoglobin, ALB : albumin, G.S. CRYST. : gamma S crystallin, BoLA : bovine leucocyte antigens, NS : Not Significant.

By combining the analysis of the three restriction enzyme patterns, was prepared a genotypic map of exon 2 of the DRB3 gene, where 4 alleles could be fully characterized (fig. 2).

The animal Z classified as susceptible is unique by the fact that it displays the 2 particular alleles we named "b" and "d". The allele "b" is characterized by a *DpnII* site toward the 5' end of exon 2 which is absent from all other resistant animals. Furthermore, animal Z, lacks in both alleles "b" and "d" an *Fnu4hI* polymorphic site toward the 3' end of exon 2.

Before reaching any conclusion, more animals need to be tested in order to investigate if the alleles found in the animal Z correlate with the character of susceptibility to dermatophilosis.

The method described here is fast, practical and can be applied to any polymorphic region displaying restriction enzyme sites.

Thus, we plan to study the polymorphism of other genes coding for proteins implicated either in immunological processes such as interferon gamma and interleukins, or in skin proteins such as keratin.

The same method will be used in other breeds such as European breeds (mainly susceptible), in tropical breeds as taurines N'Dama and Baoule, crossbreds of Guadeloupe Creole and zebus of Cameroon and Burkina Faso.

The selection of genotypes resistant or susceptible to dermatophilosis remains another alternative to tick control and in particular in areas in which tick eradication is impossible.

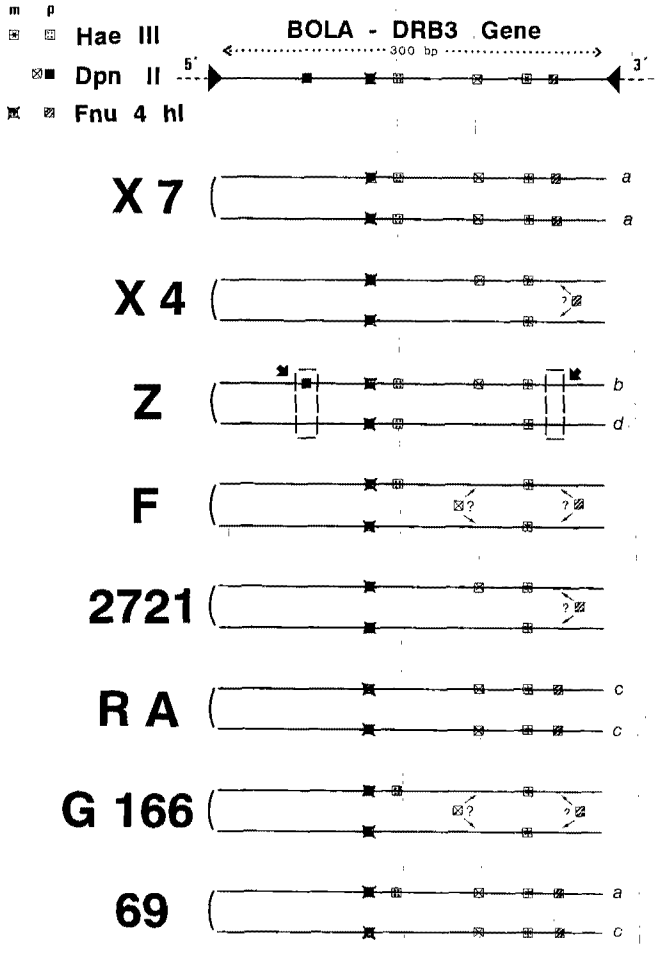

Figure 2 : Genetic map of exon 2 of the BoLA DRB3 gene encountered in the 8 Brahman cattle tested. Symbols indicate the position of restriction enzyme sites within exon 2 ; "m" and "p" design whether the site is monomorphic of polymorphic respectively. Arrows indicate the characteristics of alleles "b" and "d" differenciating the susceptible animal Z from other resistant animals. When a restriction site is ambiguous and cannot be placed in a given allele it is noted with "?".

ACKNOWLEDGEMENT

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Dermatophilosis is a disease associated with the tick *Amblyomma variegatum*, and a genetic predisposition to the manifestation of symptoms has been demonstrated. Indeed, the Creole cattle of Guadeloupe constitute a population which is highly resistant to this disease, whereas the Brahman zebu cattle of Martinique seem very susceptible. However, in this Brahman population there is a gradient regarding the severity of symptoms depending on individuals. In several herds of these pure zebu Brahman, kept under the same farming conditions, we selected susceptible and resistant groups because of the simultaneous presence of animals affected or not by clinical dermatophilosis. In these animals we studied several highly polymorphic genetic systems such as haemoglobin, albumin, the BoLA Complex (class I and II) and the gamma S crystallin gene. Only exon 2 of the BoLA-DRB3 gene, investigated by PCR-RFLP technique, showed interesting polymorphisms. We have established a genotypic map showing at least 4 different alleles of which 1 seems particular to one animal susceptible to dermatophilosis. Before reaching any conclusion further investigations with more DNA samples of susceptible animals are needed.

Key words : Brahman Zebu cattle - Dermatophilosis - Tick - *Amblyomma variegatum* - Disease resistance - Gene - Genetic polymorphism - Genetic marker - Martinique.

MAILLARD (J.C.), PALIN (C.), TRAP (I.), BENSAID (A.). Un intento para la identificación de los marcadores genéticos de la resistencia o la susceptibilidad de la dermatofilosis en la población cebuína Brahman, en Martinica. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 291-295

La dermatofilosis es una enfermedad relacionada con el ácaro *Amblyomma variegatum*, para la cuál se ha demostrado una predisposición genética a la manifestación de los síntomas. El ganado Creole de Guadalupe es altamente resistente a esta enfermedad, mientras que el cebú Brahman de Martinica parece ser muy susceptible. Sín embargo, parece existir un gradiente en la severidad de los síntomas en los diferentes individuos de esta población de Brahman. Se seleccionaron grupos de animales resistentes, en varios hatos Brahman puros, mantenidos bajo las mismas condiciones, en base a la presencia simultánea de animales afectados o no por la dermatofilosis clínica. En estos grupos se estudiaron varios sistemas genéticos altamente polimórficos, como la hemoglobina, la albúmina, el complejo BoLA (clase I y II) y el gen cristalino gama S. El único que mostró polimorfismos interesantes fue el exon 2 del gen BoLA-DRB3, estudiado mediante la técnica PCR-RFLP. Se estableció un mapa genotípico, que muestra al menos cuatro alelos diferentes, uno de los cuales parece propio de los animales susceptibles para la dermatofilosis. Antes de emitir una conclusión, deben llevarse a cabo investigaciones con más muestras de ADN de animales susceptibles.

Palabras claves : Cebú Brahman - Dermatofilosis - Garrapata - *Amblyomma variegatum* - Resistancia a las enfermedades - Gen - Polymorfismo genético - Marcador genético - Martinica.